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# Antibrowning Agents

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## **I. INTRODUCTION**

### **A. Browning as a Problem**

Many plant foods are subject to degradative reactions during handling, processing, or storage, collectively described as browning reactions, that result in the formation of brown, black, gray, or red colored pigments (Nichols, 1985; Feinberg et al., 1987). Such reactions are generally grouped into two categories: enzymatic browning and nonenzymatic browning. Examples of the former include browning of cut apples or potatoes, while examples of the latter include browning of shelf-stable, pasteurized juices and dehydrated vegetables.

Enzymatic browning results from the oxidation of polyphenols to quinones, catalyzed by the enzyme polyphenol oxidase (E.C. 1.14.18.1 and E.C. 1.10.3.1; also known as PPO, tyrosinase, *o*-diphenol oxidase, and catechol oxidase), and subsequent further reaction and polymerization of the quinones. This discoloration is generally a problem with raw fruit and vegetable products rather than blanched or thermally processed products since enzymes would be inactivated in the latter. Enzymatic browning of raw commodities may result from physiological injury; senescence; pre- or postharvest bruising; disruption of the fruit or vegetable flesh by peeling, coring, slicing, or juicing; tissue disruption from freeze-thaw cycling; and tissue disruption by bacterial growth. The occurrence of enzymatic browning can limit the shelf-life of fresh-cut fruits and salad vegetables, fresh mushrooms, prepeeled potatoes, and other fresh products of commercial importance (Huxsoll et al., 1989). This problem has held back the development and commercialization of fresh-cut fruits such as sliced apples. Enzymatic browning also may be a problem with some dehydrated and frozen fruits and vegetables (Shewfelt, 1986; Hall, 1989). In addition to causing discoloration, enzymatic browning reactions in fruit and vegetable products also

can result in loss of ascorbic acid (vitamin C) through reaction with quinones. Enzymatic browning is usually controlled by blanching, where applicable (McCord and Kilara, 1983; Hall, 1989; Ma et al., 1992); acidification; and application of sulfites (which are now subject to regulatory constraints with a number of commodities) or sulfite substitutes such as ascorbic acid or cysteine. These substitutes are generally less effective than sulfites.

Nonenzymatic browning reactions may result from the classic Maillard reaction between carbonyl and free amino groups, i.e., reducing sugars and amino acids (Hodge, 1953), which produces melanoidin pigments in a wide variety of foods including dairy, cereal, fruit, and vegetable products (Labuza and Schmidl, 1986; Handwerk and Coleman, 1988). Such discolorations generally occur in products that are subjected to heat and/or prolonged storage. Nonenzymatic browning can be minimized by avoidance of excessive exposure to heat, control of moisture content in dehydrated products, and application of sulfites. Other nonenzymatic browning reactions will be discussed in Section II.

## **B. Regulatory Issues and Other Constraints**

Sulfites have been associated with occurrence of allergic reactions, in some cases severe, with some individuals who are asthmatic. Consequently, the U.S. Food and Drug Administration has banned the use of sulfites in certain raw fruit and vegetable products. Other products such as wine and packaged dehydrated fruits and vegetables must be labeled to indicate the presence of sulfites.

While a number of sulfite substitutes exist, there is a strong reluctance on the part of some food processors to use them since a label declaration would normally be required. Many consumers seek "natural" ingredients and might be expected to reject products in which "chemicals" are used as additives. Various plant extracts and other natural products have been found to possess antibrowning activity, but there is no assurance that such products are free of toxicants, and some form of regulatory approval would probably be required before they could be used.

## **C. Scope of Review**

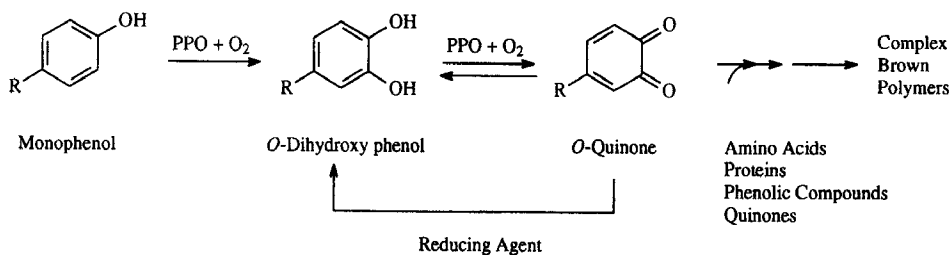
Because of the economic importance of browning reactions in foods, restrictions in the use of sulfites, and limitations in the efficacy of conventional sulfite substitutes, the search for more effective alternatives has been a very active area of research. In this chapter, we will briefly review the chemistry of browning reactions in foods and the use of sulfites as browning inhibitors. However, the main focus will be on alternatives to sulfites, especially developments in the area of new antibrowning agents and methods of application. We also will address specific browning control issues with certain commodities such as fresh-cut fruits, prepeeled potatoes, mushrooms, and fresh juices.

# **II. CHEMISTRY OF BROWNING REACTIONS IN FOODS**

## **A. Enzymatic Browning**

In this section, we will provide an overview of the reactions in foods that result in browning or other related dark discolorations. The sequence of reactions classified as enzymatic browning is usually initiated by the hydroxylation of monophenolic compounds to *o*-diphenols in the presence of atmospheric oxygen and PPO. The *o*-diphenols then undergo further oxidation, also catalyzed by PPO, to *o*-quinones. The highly reactive quinones

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**Figure 1** Enzymatic browning reaction, showing action of reducing agents as browning inhibitors. (From Sapers, 1993.)

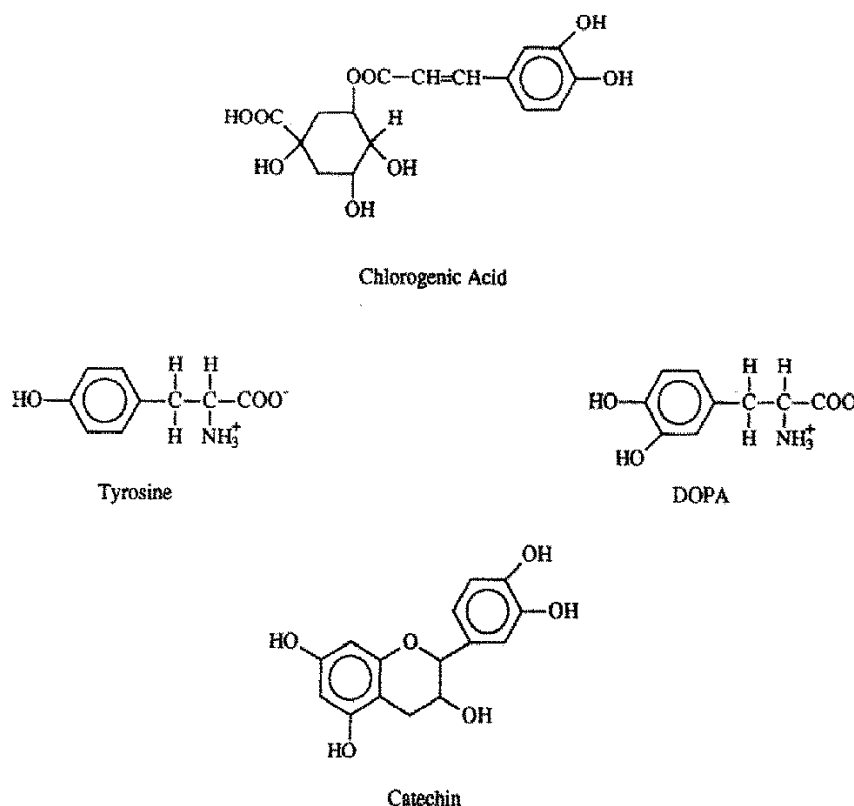
condense and react nonenzymatically with various other compounds, including phenolic compounds and amino acids, to produce pigments of indeterminate structure (Fig. 1). The subject of enzymatic browning has been reviewed by many authors, including Mayer and Harel (1979), Vamos-Vigyazo (1981, 1995), McEvily et al. (1992), Walker (1995), and Martinez and Whitaker (1995).

Enzymatic browning reactions occur in many plants, usually when plant cells are disrupted so that endogenous substrates and PPO, which are normally compartmentalized, become mixed in the presence of oxygen. Each plant species contains a characteristic pattern of phenolic compounds that are substrates of PPO. Numerous papers and reviews describing these compounds have been published (Maga, 1978; Mayer and Harel, 1979; Vamos-Vigyazo, 1981; Gross, 1981; Herrmann, 1989; Friedman, 1997). Some of the more important substrates in fruits and vegetables are catechins, chlorogenic acid, 3,4-dihydroxyphenylalanine (DOPA), and tyrosine (Fig. 2).

The optimum pH for PPO activity is between 5 and 7. PPO is relatively heat labile, and PPO-catalyzed reactions can be inhibited by acids, halides, phenolic acids, sulfites, chelating agents, reducing agents such as ascorbic acid and dithiothreitol, quinone couplers such as cysteine, and substrate binding compounds such as polyvinylpolypyrrolidone (PVPP) and  $\beta$ -cyclodextrin. With some of these compounds, the inhibitory effect is directly on PPO, but in most cases the effect is to inhibit the browning reaction by removing substrates or blocking further reaction of intermediates. A detailed examination of PPO inhibitors and mechanisms of inhibition is beyond the scope of this review since the subject has been addressed elsewhere (Pifferi et al., 1974; Mayer and Harel, 1979; Walker and McCallion, 1980; Vamos-Vigyazo, 1981; Dudley and Hotchkiss, 1989; Ferrar and Walker, 1996). However, we will examine a number of applications of PPO inhibitors as browning inhibitors for fruit and vegetable products.

## B. Nonenzymatic Browning

Nonenzymatic browning via the Maillard reaction between carbonyl and amino groups (Fig. 3) represents a complex sequence of nonenzymatic reactions that ultimately result in the formation of nitrogenous polymers and copolymers of variable composition (Hodge, 1953). In addition to discolorations associated with pigment formation, flavor changes may result from the formation of Strecker degradation aldehydes and other volatile compounds (Whitfield, 1992). Participation of free amino groups in browning reactions may result in losses of essential amino acids and reduced protein digestibility (O'Brien and Morrissey, 1989). Nonenzymatic browning reactions also can result in a loss of nutrients such as



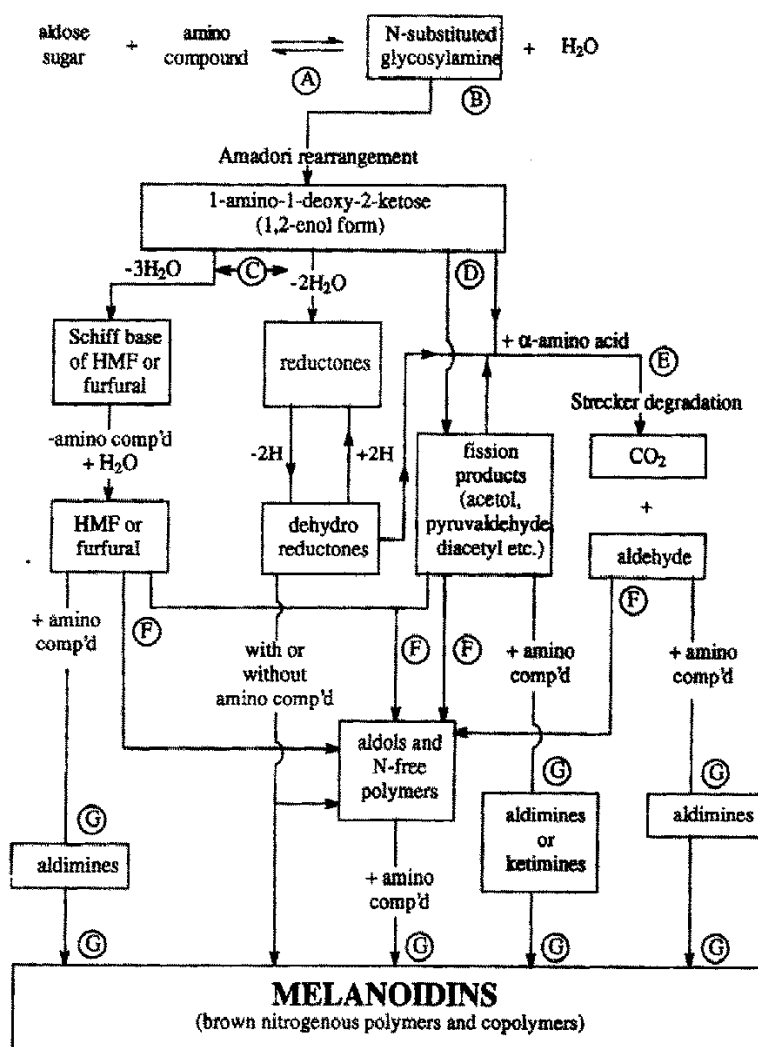
**Figure 2** Common substrates of polyphenol oxidase in fruits and vegetables.

ascorbic acid, which may become oxidized to dehydroascorbic acid, a highly unstable intermediate that reacts further via aldol condensation or reaction with amino groups to form brown pigments (Kacem et al., 1987; Wong and Stanton, 1989; Löscher et al., 1991). Browning due to anaerobic degradation of ascorbic acid is very important in processed fruit juices enriched with vitamin C. Browning also may result from sugar degradation (Lee and Nagy, 1988). These reactions have been the subject of numerous reviews (Waller and Feather, 1983; Handwerk and Coleman, 1988; Namiki, 1988) and will not be discussed further.

Other nonenzymatic reactions may result in brown or other dark discolorations. The "after-cooking darkening" reaction induced by heat during cooking or steam-peeling of potatoes is attributed to formation of iron complexes of chlorogenic acid and is controlled by addition of chelating agents such as sodium acid pyrophosphate (Smith, 1987a). Phenolic compounds can undergo nonenzymatic oxidation that results in browning (Cilliers and Singleton, 1989). We have observed browning in mushrooms treated with 0.01% sodium hypochlorite which oxidizes polyphenols to yield pigments similar to those produced by the enzymatic polyphenol oxidation (Choi and Sapers, 1994a).

Nonenzymatic browning reactions in fruit and vegetable products depend on product composition (Wong and Stanton, 1989; Kennedy et al., 1990) and pH (Wedzicha and Goddard, 1988; O'Brien and Morrissey, 1989) and is usually associated with exposure to heat during processing and storage (Nagy et al., 1990), exposure to oxygen (Kacem et

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**Figure 3** Nonenzymatic browning reaction (Maillard reaction). A, sugar-amine condensation; B, Amadori rearrangement; C, sugar dehydration; D, sugar fragmentation; E, Strecker degradation of amino acid moiety; F, aldol condensation; G, aldehyde-amine polymerization and formation of heterocyclic nitrogen compounds. (From Hodge, 1953.)

al., 1987), and insufficient moisture reduction in dehydrated products (Monsalve et al., 1990). As indicated previously, discoloration and other quality loss due to nonenzymatic browning can be prevented by treatment of fruits and vegetables with sulfites (Bolin and Steele, 1987). If sulfiting is not permitted, there are few options for controlling browning other than reduced exposure to high temperatures, better control of water activity (Labuza and Saltmarch, 1981), and in some cases removal of reducing sugars by treatment with glucose oxidase (Low et al., 1989) or manipulation of raw material storage conditions, e.g., potatoes for chipping (Smith, 1987b). Sulfhydryl-containing amino acids show some capacity to inhibit nonenzymatic browning (Friedman and Molnar-Perl, 1990), but such treatments must have regulatory approval and cannot introduce atypical flavors to treated

products. Bolin and Steele (1987) reported that cysteine was ineffective in controlling browning of dried apple.

### **III. SULFITES AS BROWNING INHIBITORS**

#### **A. Treatment of Foods with Sulfites**

Sulfites are unique in their ability to perform a number of useful functions as food additives—control of both enzymatic and nonenzymatic browning, suppression of microbial growth, and bleaching (Taylor et al., 1986). They have been used since antiquity for these and other purposes. In the case of enzymatic browning, sulfites act as PPO inhibitors and also react with intermediates to prevent pigment formation (Sayavedra-Soto and Montgomery, 1986). Sulfites inhibit nonenzymatic browning by reacting with carbonyl intermediates, thereby blocking pigment formation (Wedzicha, 1987).

Treatment conditions vary widely. Sulfite may be applied as sulfur dioxide; sulfurous acid; or sodium (or potassium) sulfite, bisulfite, or metabisulfite. Treatment levels vary widely, but treatment residues usually do not exceed several hundred ppm, although some products may contain 1000 ppm (Taylor et al., 1986). Among the products that are treated with sulfites are dehydrated fruits and vegetables, prepeeled potatoes, fresh grapes, and wine. Maximum levels of 300, 500, and 2000 ppm have been proposed for fruit juices, dehydrated potatoes, and dried fruit, respectively (FDA, 1988b).

#### **B. Safety and Regulatory Issues**

Sulfite residues in foods have been responsible for some severe allergic reactions in susceptible individuals, usually asthmatics. Fatal anaphylactic reactions have been reported (Taylor et al., 1986). The FDA has restricted use of sulfites in certain categories of foods where there is no means of alerting sensitive consumers to their presence (FDA, 1986). Fruit and vegetable products that are consumed raw and sold unlabeled in salad bars, restaurants, or from bulk containers so that the consumer cannot be alerted to their presence fall in this category (FDA, 1986). However, a ban on sulfiting prepeeled potatoes, a product usually sold to the food service industry (FDA, 1990), was vigorously opposed by the prepeeled potato industry on the ground that there were no effective substitutes, and the ban was eventually overturned in the courts on a technicality (Anonymous, 1990). FDA established labeling requirements for foods containing sulfites and affirmed the GRAS status of sulfiting agents in 1988 (FDA, 1988a, 1988b).

Fear that use of sulfites as browning inhibitors for fruit and vegetable products might be restricted prompted the food industry to seek alternatives. During the 1980s, this was an area of great research activity, and a number of new browning inhibitors were developed (Sapers and Hicks, 1989). In recent years, the number of papers in this area has diminished somewhat, but there is still commercial interest in developing improved sulfite substitutes for important commodities such as fresh-cut apples and potatoes. Use of benign ingredients as browning inhibitors and treatments that leave no residues have been especially prominent. In part this is driven by the reluctance of consumers to buy products containing "chemicals" of any kind.

In the remainder of this chapter, we will review the range of antibrowning agents that have been considered, including some of the newer developments in this area.

### IV. ALTERNATIVES TO SULFITES

The search for alternatives to sulfite has been complicated by the fact that sulfites are extremely potent as browning inhibitors, inexpensive to use, and multifunctional, exhibiting antimicrobial activity as well as anti-browning activity. Ideally, sulfite substitutes should exhibit similar properties. Moreover, sulfite substitutes should be safe and free of regulatory constraints. Unfortunately, most alternatives to sulfites do not meet these criteria, so that the choice of agent must represent a compromise. Furthermore, since individual anti-browning agents may be deficient in some respects, most alternatives to sulfite represent combinations of agents that act synergistically or at the very least have an additive effect. Even so, there is no “magic bullet” to control browning in commercial use or under development. Perhaps the eventual development of genetically engineered commodities lacking PPO or with reduced levels of this enzyme will eliminate the need for browning inhibitors. For the foreseeable future, however, there will be a need for safe and cost-effective anti-browning agents.

#### A. Conventional Alternatives to Sulfite

##### 1. Ascorbic Acid–Based Formulations

Ascorbic acid (vitamin C) has been used as an antibrowning agent for more than five decades and is still the most widely used alternative to sulfiting agents. This may be an outgrowth of the common kitchen practice of using lemon juice to delay browning during food preparation. The earliest scientific studies were reported by Tressler and DuBois (1944) and Esselen et al. (1945). These pioneering investigators added ascorbic acid or its isomer erythorbic (*d*-isoascorbic) acid to syrups or dips to control browning of fresh sliced and frozen apples and peaches. In most respects, ascorbic and erythorbic acids are similar in activity as antioxidants (Borenstein, 1965) and browning inhibitors (Sapers and Ziolkowski, 1987). However, the latter compound does not have vitamin C activity. In some cases, an organic acid such as citric acid and a firming agent such as calcium chloride were added along with the ascorbic acid. To improve the uptake of these agents and at the same time remove oxygen from the product void spaces, vacuum infiltration was sometimes used in conjunction with browning inhibitor treatment (Guadagni, 1949). More recently, Sapers et al. (1990) reported that the “water-logging” effects seen in vacuum-infiltrated fresh apples could be avoided by infiltrating ascorbate or erythorbate solutions under pressure.

The chemical basis for the efficacy of ascorbic acid treatments is the ability of ascorbic acid to reduce quinones, produced by PPO-catalyzed oxidation of polyphenols, back to dihydroxy polyphenols. As long as quinones do not accumulate, further reactions leading to pigment formation are avoided. When the added ascorbic acid is depleted quinones will accumulate, and browning will result. Thus, the primary effect of ascorbic acid is as an inhibitor of the enzymatic browning reaction, not as an inhibitor of PPO per se. However, ascorbic acid does have some direct inhibitory effect against PPO (Vamos-Vigyazo, 1981). Dehydroascorbic acid, the oxidation product of ascorbic acid that is formed during quinone reduction, can itself undergo nonenzymatic browning, leading to product discoloration. We have seen such discolorations during refrigerated storage of fresh apple and pear juices that were treated with ascorbic acid during juicing as a temporary means to suppress browning and then rapidly filtered or centrifuged to remove particu-

late-bound PPO. This treatment permanently eliminates the capacity of juices to undergo enzymatic browning (See Section V) (Sapers, 1991).

During the 1980s, many ascorbic acid-based browning inhibitor formulations were marketed. A survey conducted by the National Restaurant Association in 1986 identified 13 suppliers of such products. These formulations usually contained ascorbic or erythorbic acid (or their sodium salts) in combination with adjuncts such as citric acid or some other acidulant, a calcium salt for firming, a phosphate such as sodium acid pyrophosphate (a chelating agent), sodium chloride, cysteine, and a preservative such as sodium benzoate or potassium sorbate (Sapers, 1993). Different formulations usually were provided for apples, potatoes, and salad vegetables. Use levels for these products varied over a wide range, and there appeared to be no consensus as to optimal ascorbic acid levels. These products were claimed to provide a shelf-life of 4–7 days under refrigeration, far less than could be achieved with sulfites.

A longer shelf-life could be achieved by shipping peeled potatoes in a preservative solution after treatment with browning inhibitors (Santerre et al., 1991). However, the logistics of this approach are highly unfavorable. Another approach to shelf-life extension was use of vacuum packing after browning inhibitor treatment (Langdon, 1987). However, rapid browning would ensue once the vacuum was broken. Vacuum packing of raw potatoes might be hazardous because of the potential ability of *Clostridium botulinum* to grow and produce toxin in peeled, partially cooked potatoes under anaerobic conditions (Tamminga et al., 1978). A recent study by Juneja et al. (1998) demonstrated growth without concurrent spoilage by *Listeria monocytogenes* in inoculated vacuum-packed potatoes under conditions of temperature abuse.

Research on ascorbic acid-2-phosphates and fatty acid esters as stabilized forms of ascorbic acid showed that these derivatives exhibited some advantages over ascorbic acid for control of browning in cut apples (Sapers et al., 1989b), cut potatoes (Sapers and Miller, 1992), and fresh fruit juice (Sapers et al., 1989b). However, no commercial interest in this technology was forthcoming, probably because of the need for regulatory approval.

At the present time, major suppliers of ascorbic acid or erythorbic acid-based browning inhibitor formulations are Monsanto Chemical Company (Snow Fresh™), EPL Technologies, Inc. (Potato Fresh™), and Mantrose-Hauser Co., Inc. (NatureSeal™). Numerous companies supply the ingredients from which such formulations can be made.

## 2. Cysteine

The ability of cysteine to inhibit enzymatic browning is well established and has been used commercially for a number of years (Cherry and Singh, 1990). This alternative to sulfites is a key ingredient of browning inhibitor formulations for apples and prepeeled potatoes supplied by EPL Technologies, Inc. (Brereton and Sapers, 1997). Cysteine is also a component of browning inhibitor treatments developed by Senesi and Pastine (1996) and by Gunes and Lee (1997). Cysteine reacts with quinone intermediates, formed by PPO-catalyzed oxidation of polyphenols, to yield stable, colorless compounds, thereby blocking pigment formation (Dudley and Hotchkiss, 1989). Cysteine also directly inhibits the enzyme (Robert et al., 1996). We have observed that cysteine-based inhibitors are particularly effective against browning of apple core tissue. However, under some conditions, cut pears and potatoes treated with cysteine show pink discolorations. At high treatment levels, a noticeable sulfury odor can result. Studies by Molnar-Perl and Friedman (1990 a,b) and Friedman (1994) suggest that reduced glutathione and N-acetylcysteine



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are nearly as effective as sulfites in controlling browning of apple, potato, and fresh fruit juices. However, only cysteine is approved for food use.

### **3. 4-Hexylresorcinol**

This PPO inhibitor is used commercially to control discoloration of unpeeled shrimp (Everfresh™) and is highly effective as a browning inhibitor for some fruits and vegetables (McEvily et al., 1991; Monsalve-Gonzalez et al., 1993; Luo and Barbosa-Canovas, 1997). While 4-hexylresorcinol has a long history of human consumption (Frankos et al., 1991), it is only approved for use on shrimp (where the application is to the peel, which is not usually eaten). Regulatory approval for other applications where it might be consumed in quantity is uncertain. We have found that it is particularly effective against core browning in fresh-cut pears (Sapers and Miller, 1998). However, 4-hexylresorcinol caused darkening and tissue breakdown in fresh mushrooms at concentrations greater than 50 ppm (Sapers et al., 1994).

## **B. Other PPO Inhibitors**

Various PPO inhibitors have been proposed as alternatives to sulfites, but they lack regulatory approval at this time. Walker and Wilson (1975) investigated inhibition of apple PPO by a number of phenolic acids. Walker (1976) reported that cinnamic acid at concentrations greater than 0.5 mM prevented browning of Granny Smith apple juice for 7 hours. Experiments carried out with cinnamate in our laboratory with cut apples also showed short-term browning inhibitor activity, but the treatment appeared to induce browning after 24 hours at ambient temperature (Sapers et al., 1989b). Benzoate also induced browning in cut apples after prolonged storage.

Kojic acid [5-hydroxy-2-(hydroxymethyl)- $\gamma$ -pyrone] has been considered for use as an alternative to sulfite. This compound can reduce quinones to polyphenols, thereby preventing browning by the same mechanism as ascorbic acid (Chen et al., 1991). It is not clear whether this compound acts as a true PPO inhibitor. The regulatory status of kojic acid is in doubt because of reported mutagenic activity (Wei et al., 1991).

Tong and Hicks (1991, 1993) reported that carrageenans and other sulfated polysaccharides showed browning inhibitor activity in apple juice and diced apples; citric acid acted synergistically with these compounds in inhibiting browning. The mechanism of browning inhibition by the sulfated polysaccharides is not known. Xu et al. (1993) reported that maltodextrin inhibited browning of ground apple.

Natural PPO inhibitors have been found in honey (Oszmianski and Lee, 1990), pineapple (Lozano de Gonzalez et al., 1993; Wen and Wrolstad, 1998), fig latex (McEvily, 1991), and a large number of botanical products (Choi et al., 1997). In some cases, the natural origin of such products has been equated with safety. This view is probably misguided since their purity and composition may be unknown, and consumption patterns of these products as browning inhibitors may be substantially different than their use as herbs or traditional medicinal agents. In any event, FDA approval would be required if the products were to be isolated, concentrated, and applied to foods as browning inhibitors.

## **C. Complexing Agents**

In studies carried out in our laboratories and in France,  $\beta$ -cyclodextrin was found to be an effective browning inhibitor for fruit and vegetable juices (Sapers et al., 1989b; Hicks

et al., 1990; Billaud et al., 1995; Hicks et al., 1996). Treatment with  $\beta$ -cyclodextrin did not control browning of cut apple or potato (Sapers et al., 1989b).  $\beta$ -cyclodextrin has been shown to form inclusion complexes with chlorogenic acid and presumably other PPO substrates, thereby effectively removing them from contact with PPO in fresh juices (Irwin et al., 1994). Although there has been commercial interest in this antibrowning agent, regulatory approval has not been forthcoming. Fruit and vegetable juices treated with insoluble polymerized forms of  $\beta$ -cyclodextrin resist browning indefinitely (Hicks et al., 1996). Use of such forms of this compound should avoid regulatory issues associated with treatment residues.

PVPP, an insoluble product used as a fining agent for juices, can bind the polyphenol substrates of PPO and thus prevent browning (Van Buren, 1989). PVPP can be separated from juices by decanting or centrifugation so that there are no treatment residues. Studies in our laboratory have shown that this agent is highly effective in preventing browning of tropical fruit juice blends (Essa and Sapers, 1998).

Sodium acid pyrophosphate (SAPP) is widely used as a chelating agent in potato products. Its primary purpose is to prevent after-cooking darkening by chelating ferrous ion, thus preventing formation of dark-colored iron-chlorogenic acid complexes (Feinberg et al., 1987). We have used it in browning inhibitor formulations for prepeeled potatoes (Sapers and Miller, 1995).

Another complexing agent, a proprietary polyphosphate marketed as Sporix, was studied in our laboratory as a potential sulfite substitute. This product is highly acidic and is reputed to be a powerful chelating agent (Friedman, 1986; Gardner et al., 1991). We found that this product was highly effective in controlling browning of cut apple and apple juice when used in combination with ascorbic acid (Sapers et al., 1989b). However, further studies with Sporix were curtailed since the supplier was unable to obtain regulatory approval for its use.

While polyphosphates and cyclodextrins each provide browning inhibition in fresh juices, use of these complexing agents in combination leads to even more effective (synergistic) results (Hicks et al., 1996). Combinations of  $\beta$ -cyclodextrin with either SAPP, Sporix, sodium hexametaphosphate, or phytic acid resulted in greater browning inhibition than predicted from the effects of each component tested separately. This synergistic effect allows greater inhibition of browning and use of lower levels of additives to achieve control of browning.

## **V. SPECIAL PROBLEMS IN CONTROL OF ENZYMATIC BROWNING**

The efficacy of browning inhibitor treatments for fruit and vegetable products sometimes depends on factors other than the antibrowning agent and application conditions used. In some cases, commodity condition plays a key role in determining treatment efficacy. In other cases, the treatment may exert an adverse effect on some quality attribute, necessitating treatment modification. In all cases, one should take a holistic approach in developing browning inhibitor treatments so that treatments are effective and avoid unforeseen adverse consequences. The following examples illustrate some of these complicating factors and strategies used to circumvent them.

### **A. Fresh-Cut Apples**

In earlier work, we found that apple plugs and slices responded better to treatment with neutral solutions of sodium ascorbate or sodium erythorbate than to treatment with the

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respective acids (Sapers, 1988; Sapers et al., 1990). Laboratory studies with fresh-cut apples demonstrated effective control of enzymatic browning by application of dips containing 2–4% sodium erythorbate and 0.1–0.2% calcium chloride. Products were packaged in films that produced a modified atmosphere with reduced oxygen and elevated carbon dioxide during storage. The modified atmosphere suppressed both browning and microbial spoilage. Typically, products had a shelf-life of 2 weeks at 4°C, based on absence of browning and microbial spoilage.

Two factors complicated this treatment. Apple slices prepared by hand in the laboratory were generally free of residual core tissue and showed uniform response to treatment. However, field tests of the treatment with commercially cored and sliced apples containing residual core demonstrated that the core tissue did not respond as well to antibrowning agents as the flesh portion of slices. Thus, slices with residual core tissue showed conspicuous browning when most slice surfaces were still free of discoloration. Research is in progress to address this problem by use of alternative antibrowning agents and modified treatment conditions (Brereton and Sapers, 1997).

The second complicating factor was the development of atypical, pineapple-like odors during modified atmosphere storage of treated products. We attribute this quality defect to the development of near-anaerobic conditions within the packages that caused apple slices to undergo anaerobic respiration and generate ethanol (Zhuang et al., 1998). We speculate that the ethanol reacted with endogenous esters on the cut surfaces of slices to produce some atypical ethyl esters by transesterification. The resulting change in volatile composition was perceived as pineapple-like. This problem could be addressed by using a packaging material with greater oxygen permeability so that an anaerobic condition would not occur during normal product storage (Brereton and Sapers, 1997). However, this represents a tradeoff since the greater oxygen concentration within the package resulted in more browning and a shorter shelf-life.

### **B. Fresh-Cut Pears**

In many respects, fresh-cut pears are similar to apples in their response to browning inhibitor treatments (Sapers and Miller, 1998). Neutral sodium ascorbate or erythorbate treatments with added calcium chloride were more effective than acidic treatments. Residual core tissue on slices did not respond as well to antibrowning treatments as the flesh portion. However, with fresh-cut d'Anjou and Bartlett pear slices, blackening of the cut edge of the skin during storage was even more conspicuous. Residual core browning could be controlled by addition of 50–100 ppm 4-hexylresorcinol to the browning inhibitor dip.

By far the most important factor affecting browning of fresh-cut pears was raw material ripeness. We found that slightly under-ripe pears responded better than fully ripe pears to application of browning inhibitor dips in combination with modified atmosphere packaging. The less ripe fruit showed less browning of cut surfaces, residual core tissue, and the cut edge of the skin than fully ripe pears. The slightly under-ripe fresh-cut pears probably suffered less tissue damage than the riper fruit during slicing. Use of slightly under-ripe fruit resulted in slightly lower flavor levels but better retention of firm texture during storage.

### **C. Pre-Peeled Potatoes**

In studies with prepeeled potatoes, we found that enzymatic browning could be controlled by treatment with ascorbic acid–2-phosphates (Sapers and Miller, 1992) or by surface

digestion with sodium hydroxide prior to application of an ascorbic acid-based browning inhibitor (Sapers and Miller, 1993). Good control of browning also could be obtained when acidic browning inhibitor solutions containing ascorbic and citric acids were applied to peeled potatoes at 45–55°C (Sapers and Miller, 1995). However, such treatment resulted in case-hardening of potato surfaces which interfered with mashing and slicing. Similar effects, although not as severe, were observed when peeled potatoes were treated with conventional acidic sulfite substitutes. We believe that this effect was due to activation of pectinmethylesterase during treatment which partially demethylated pectin, creating new binding sites for calcium or magnesium ions. Subsequent cross-linking of pectin by these ions would result in the atypical surface firming that we observed (Sapers et al., 1997).

The textural abnormality could not be addressed by reducing the treatment temperature or by using a neutral browning inhibitor treatment since these steps would have greatly reduced browning inhibitor efficacy. However, by buffering the browning inhibitor solution, a compromise treatment was devised that yielded an extended product shelf-life without the textural defect (Martin et al., 1997).

#### **D. Washed Fresh Mushrooms**

Another example of a factor interfering with browning inhibitor application was seen with washed, fresh mushrooms. It is well-known that washing predisposes fresh mushrooms to premature spoilage by *Pseudomonas tolaasii*, which produces a dark, sunken lesion on mushroom surfaces called “brown” or “bacterial blotch.” Washing with sulfite solution avoided this problem because of sulfite’s antimicrobial activity and, at the same time, inhibited browning of the mushroom surface. However, sulfites can no longer be used on fresh mushrooms. Washing with chlorinated water sometimes caused mushroom darkening (Choi and Sapers, 1994a) or induced purple discolorations on mushroom surfaces, attributed to the reaction between L-DOPA oxidation products and sinapic acid (Choi and Sapers, 1994b). We were unable to find an effective alternative to sulfite for controlling browning of fresh mushrooms until we addressed the microbiological spoilage problem first. We found that a wash in 5% hydrogen peroxide to reduce the bacterial load prior to application of a sodium erythorbate spray inhibited browning with no shelf-life penalty due to microbial spoilage (Sapers et al., 1994, 1995).

These examples clearly show the fallacy of looking at browning inhibitor treatments in isolation. One must consider the tendency of the commodity and even specific cultivars to undergo browning; commodity maturity or ripeness; raw material condition; and peripheral effects of treatment on product quality or susceptibility to microbial spoilage. Therefore, antibrowning agents should be tested with a broad range of raw material types, prepared in a form representative of commercial practice, stored under realistic conditions, and evaluated for overall quality as well as efficacy in controlling browning.

#### **E. Fresh Juices**

The growing popularity of minimally processed fruit and vegetable products is reflected in the appearance of supermarket juice bars and other retail establishments selling beverages based on fresh fruit homogenates (“smoothies”) and juice blends. Such products may contain active enzymes, including PPO, which could affect product color with some commodities such as pear, apple, and mango. Recent concern over the microbiological safety of unpasteurized cider will undoubtedly restrict production and marketing of some

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products, but several nonthermal pasteurization treatments under development (i.e., membrane filtration, ultra-high pressure, pulsed electric fields, UV-pasteurization, high intensity pulsed light) may provide a means of safely preserving fresh juice products. In most cases, these treatments do not inactivate enzymes, and products derived from commodities containing sufficient PPO may undergo browning during storage. Browning inhibition treatments entailing use of additives may not be acceptable to the fresh juice market.

We have developed technology for controlling browning in fresh juices without use of additives that may be applicable to this situation. In some unfiltered juices such as apple and pear, PPO is bound to particulate fractions and can be removed by filtration or centrifugation to yield a nonbrowning product (Sapers, 1991). Removal of PPO can be enhanced by addition of insoluble fining agents such as diatomaceous earth, bentonite, or chitosan (Sapers, 1991, 1992). Alternatively, an insoluble adsorbent or complexing agent such as polymerized  $\beta$ -cyclodextrin (Hicks et al., 1990, 1996) or PVPP (Essa and Sapers, 1998) could be added to remove PPO substrates from the juice. These treatments leave no residues in the juice but might adsorb or otherwise remove components important to juice quality such as flavor compounds, pigments, and suspended solids that contribute to desirable cloud. These potential problems as well as treatment efficacy, cost, and regulatory status must be considered in determining the feasibility of technology to control browning in fresh juices without use of additives.

### **F. Lettuce**

Harvested lettuce is subject to enzymatic browning of the cut stem (butt discoloration). Peiser et al. (1998) demonstrated that wounding of lettuce during cutting induced production of phenylalanine ammonia lyase (PAL) and subsequent formation of phenolic substrates of PPO by the phenylpropanoid pathway, which, upon oxidation, caused browning. Application of acetic acid to stem tissue completely inhibited PAL activity and the production of wound-induced phenolics, thereby preventing browning (Tomas-Barberan et al., 1997). Castaner et al. (1997) found that acetic and propionic acid treatments were effective in reducing butt discoloration during storage and commercial handling. Heat shock treatments also suppressed PAL activity; synthesis of chlorogenic acid, dicaffeoyl tartaric acid, and isochlorogenic acid; and subsequent browning (Loaizavelarde et al., 1997). These studies demonstrate the potential value of treatments that interfere with the formation of PPO substrates as an alternative to the direct inhibition of PPO or addition of reducing or complexing agents that block pigment formation.

### **G. Use of Nonbrowning Cultivars**

Control of enzymatic browning without use of antibrowning agents might be achieved in some commodities by use of raw materials that are deficient in PPO. Some cultivars are naturally deficient in PPO or PPO substrates so that they are less subject to browning than more common cultivars (Coseting and Lee, 1987; Sapers and Douglas, 1987; Sapers et al., 1989a). Nonbrowning cultivars might be developed through breeding programs (Woodwards and Jackson, 1985) or genetic engineering (Bachem et al., 1994; Mooibroek et al., 1996). However, such cultivars should be equivalent to conventional cultivars in quality, processability, disease resistance, yield, and other important characteristics. Whether the cost of eliminating the browning trait by genetic engineering can be justified remains to be seen.

## VI. CONCLUSIONS

Recent advances in antibrowning agents and improved application methods, used in conjunction with antimicrobial treatments and modified atmosphere packaging, have yielded large improvements in control of browning in fresh-cut and other minimally processed fruit and vegetable products. However, whether such improvements can be translated into shelf-life extension will depend on the retention of other quality attributes such as flavor and texture as well as on the suppression of microbial spoilage. Of course, we must always be alert to the possibility that suppression of spoilage organisms might create a niche for human pathogens.

Technical achievements in development of antibrowning agents are only part of the story. Regulatory hurdles, labeling issues, lack of competitive advantage, and cost may limit the commercialization of promising treatments. Thus, there is a continuing need for research to develop effective antibrowning treatments that meet the requirements of technical and economic feasibility, safety, and consumer acceptance.

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